Amendments to the Specification

Please replace the paragraph beginning at page 11, line 26 with the following amended paragraph:

Isoforms of BNP can be obtained by extraction from a natural source (e.g., from isolated cells, tissues or bodily fluids), by expression of a recombinant nucleic acid encoding the polypeptide, or by chemical synthesis. For example, standard recombinant technology using expression vectors encoding isoforms of BNP (as described below) can be used. The resulting polypeptides then can be purified using, for example, affinity chromatographic techniques and HPLC. The extent of purification can be measured by any appropriate method, including but not limited to: column chromatography, polyacrylamide gel electrophoresis, or high-performance liquid chromatography. Isoforms of BNP can be "engineered" to contain a tag sequence that allows the polypeptide to be purified (e.g., captured onto an affinity matrix). For example, a tag such as c-myc, hemagglutinin, polyhistidine, or ~~Flag™~~ FLAG™ epitope tag (Kodak) can be used to aid polypeptide purification. Such tags can be inserted anywhere within the polypeptide including at either the carboxyl or amino termini. Other fusions that can be used include enzymes that aid in the detection of the polypeptide, such as alkaline phosphatase.

Please replace the paragraph beginning at page 21, line 23 with the following amended paragraph:

In immunological assays, an antibody having specific binding affinity for an isoform of BNP or a secondary antibody that binds to such an antibody can be labeled, either directly or indirectly. Suitable labels include, without limitation, radionuclides (e.g., ^{125}I, ^{131}I, ^{35}S, ^{3}H, ^{32}P, ^{33}P, or ^{14}C), fluorescent moieties (e.g., fluorescein, FITC, PerCP, rhodamine, or PE), luminescent moieties (e.g., ~~Qdot™~~ QDOT™ nanoparticles supplied by the Quantum Dot Corporation, Palo Alto, CA), compounds that absorb light of a defined wavelength, or enzymes (e.g., alkaline phosphatase or horseradish peroxidase). Antibodies can be indirectly labeled by conjugation with biotin then detected with avidin or streptavidin labeled with a molecule described above. Methods of detecting or quantifying a label depend on the nature of the label and are known in the art. Examples of detectors include, without limitation, x-ray film, radioactivity counters, scintillation counters, spectrophotometers, colorimeters, fluorometers, luminometers, and

densitometers. Combinations of these approaches (including "multi-layer" assays) familiar to those in the art can be used to enhance the sensitivity of assays.

Please replace the paragraph beginning at page 27, line 24 with the following amended paragraph:

Another FRET format utilizes ~~TaqMan®~~ TAQMAN® gene expression technology to detect the presence, absence, or level of an amplification product, and hence, the presence, absence, or level of nucleic acid encoding an isoform of BNP. ~~TaqMan®~~ TAQMAN® gene expression technology utilizes one single-stranded hybridization probe labeled with two fluorescent moieties. When a first fluorescent moiety is excited with light of a suitable wavelength, the absorbed energy is transferred to a second fluorescent moiety according to the principles of FRET. The second fluorescent moiety is generally a quencher molecule. During the annealing step of the PCR reaction, the labeled hybridization probe binds to the target DNA (i.e., the amplification product) and is degraded by the 5' to 3' exonuclease activity of the Taq Polymerase during the subsequent elongation phase. As a result, the excited fluorescent moiety and the quencher moiety become spatially separated from one another. As a consequence, upon excitation of the first fluorescent moiety in the absence of the quencher, the fluorescence emission from the first fluorescent moiety can be detected. By way of example, an ABI PRISM® 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) uses ~~TaqMan®~~ TAQMAN® gene expression technology, and is suitable for performing the methods described herein for detecting isoforms of BNP. Information on PCR amplification and detection using an ABI PRISM® 7700 system can be found at the website of Applied Biosystems.

Please replace the paragraph beginning at page 28, line 23 with the following amended paragraph:

As an alternative to FRET, amplification product can be detected using, for example, a fluorescent DNA binding dye (*e.g.,* ~~SYBRGreenI®~~ SYBRGREENI® nucleic acid dye or ~~SYBRGold®~~ SYBRGOLD® nucleic acid dye (Molecular Probes)). Upon interaction with an amplification product, such DNA binding dyes emit a fluorescent signal after excitation with light at a suitable wavelength. A double-stranded DNA binding dye such as a nucleic acid

intercalating dye also can be used. When double-stranded DNA binding dyes are used, a melting curve analysis usually is performed for confirmation of the presence of the amplification product.

Please replace the paragraph beginning at page 29, line 24 with the following amended paragraph:

It was determined that intron 2 of the BNP genomic sequence encoded a polypeptide having homology to the C-terminal of the snake DNP polypeptide. To determine if the polypeptide encoded by intron 2 was expressed, Northern blotting was performed using cellular RNA isolated from kidney, brain, and heart (left ventricles and atria) tissue of patients with and without heart failure. Approximately 5 μg of RNA was electrophoresed through a 1% agarose gel containing formaldehyde and transferred to a nylon membrane. Northern blotting was performed using the ~~NorthernMax™~~ NORTHERNMAX™ hybridization kit from Ambion, Inc. (Austin, TX), according to the manufacturer's instructions. The membranes were UV autocross-linked, prehybridized at 42°C for 1 hour, and hybridized at 42°C overnight with a labeled probe containing the second intron sequence. The probe was labeled with ^{32}P using the Random Primers DNA Labeling System (Gibco BRL). The membranes were washed twice in low stringency buffer (provided in ~~NorthernMax™ kit~~ NORTHERNMAX™ hybridization kit, equivalent to 2X SSC, 0.1% SDS or 2X SSPE, 0.1% SDS) at room temperature, five minutes per wash, then washed twice in a high stringency buffer (provided in ~~NorthernMax™ kit~~ NORTHERNMAX™ hybridization kit, equivalent to 0.1X SSC, 0.1% SDS or 0.1X SSPE, 0.1% SDS) at 42°C, 15 minutes per wash. Hybridization signals on the blots were analyzed by autoradiography. Message for the second intron was detected in the left atrium of heart failure patients.

Please replace the paragraph beginning at page 30, line 13 with the following amended paragraph:

Total RNA was isolated from normal and heart failure human heart tissue using ~~TRIzol®~~ TRIZOL® Reagent (Gibco BRL), a monophasic solution of phenol and guanidine isothiocyanate. Complementary DNA (cDNA) was produced using an oligo(dT) primer and the ~~ThermoScript™~~ THERMOSCRIPT™ RT-PCR Kit from Invitrogen Corp. (Carlsbad, CA) according to the

manufacturer's instructions. RT-PCR was performed using a forward primer having the sequence: 5'-AGACATGGATCCCCAGACAG-3' (SEQ ID NO:21; 5' start codon is underlined) and a reverse primer having the sequence: 5'-CAAGAGGAAGCGATGTCCAG-3' (SEQ ID NO:22, positioned at nucleotides 113 to 133 in the second intron). After combining the cDNA and primers, an initial denaturation was performed at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 secs, annealing at 52°C for 1 min, and elongation at 72°C for 45 sec then a final extension at 72°C for 10 min. The resulting PCR product (~520bp hBNP2 cDNA) was subcloned into Topo vector and sequenced for confirmation. The nucleic acid sequence of the hBNP2 cDNA is shown in FIG. 1A.

Please replace the paragraph beginning at page 30, line 28 with the following amended paragraph:

Total RNA was isolated from normal and heart failure dog heart tissue using ~~TRIzol®~~ TRIZOL® Reagent (Gibco BRL), a monophasic solution of phenol and guanidine isothiocyanate. cDNA was made using an oligo(dT) primer and the ~~ThermoScript™~~ THERMOSCRIPT™ RT-PCR Kit from Invitrogen Corp. (Carlsbad, CA) according to the manufacturer's instructions. RT-PCR was performed using a forward primer having the sequence: 5'-TTCTCTCCAGCGACATGGAG-3' (SEQ ID NO:23, 5' start codon is underlined) and a reverse primer having the sequence: 5'-GGACTCTTTCTGCTCCAAGG-3' (SEQ ID NO:24, positioned at nucleotides 288 to 308 in the second intron). After combining the cDNA and primers, an initial denaturation was performed at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 secs, annealing at 55°C for 1 min, and elongation at 72°C for 45 sec then a final extension at 72°C for 10 min. The resulting PCR product (~540bp dBNP2 cDNA) was subcloned into Topo vector and sequenced for confirmation. The nucleic acid sequence of the dBNP cDNA is shown in FIG. 1B.